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Interaction of various pectin formulations with porcine colonic tissues ☆

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Abstract

Pectins of low and high degrees of esterification, as well as pectin derivatives carrying primary amines, were investigate for gel forming ability with mucosal tissues. The combination of scanning electronic microscopy and small deformation dynamic mechanical studies revealed that pectins with higher net electrical charges are more bioadhesive than the less charged ones. Both the negatively charged pectin formulation, P-25, and the positively charged formulation, P-N, were able to synergize with the mucus to produce rheologically strengthened gels. The highly esterified pectin, P-94, also synergized with the mucosal glycoproteins to form a gel structure via coil entanglements. The ex vivo studies further confirmed the microstructures of mucus gel networks with adsorbed pectins. When incubated with porcine intestinal mucus membrane, P-94 gels were found generally bound to the lumen area, P-25 gels were able to penetrate deeply near the wall area, P-N gels interacted with mucins via electrostatic bonding and dispersed into the whole area from the lumen to the wall. Hence, both P-N and P-94, by enhancing the protective barrier properties of mucus systems, may be useful alternatives for the treatment of mucus related irritation and infection. In drug-delivery systems, P-N and P-25 would deliver incorporated drugs mainly by pectin dissolution, while a diffusion mechanism would release drugs from P-94 gels.

Keywords: Pectin; Mucin; Drug delivery; Bioadhesiveness

1. Introduction

Pectin, a plant polysaccharide, recently has gained increasing research interest in gastroenterological medicine, for use in drug carriers for oral drug delivery [1–4] and natural therapeutics for diseases related to irritation of mucous membranes [5]. Furthermore, pectin is used as a physical barrier for the protection of epithelium against opportunistic microbial invasion during stress [6]. Among many responses of pectin to the gastrointestinal tract (GIT), the interaction with mucus is the subject of intensive studies [7–9]. It has been demonstrated that pectin bound to mucin, the main constituent of mucus in GIT, forms interpenetrating gel networks [5]. Other mucoadhesive polymers of similar properties include polyacrylic acid (PAA), carboxymethyl cellulose (CMC), hyaluronic acid (HA), chitosan and gelatin.

A number of mechanisms have been suggested to explain the interaction of polymers with mucus. Molecular interpenetration is the most accepted one, by which physical entanglement, hydrogen bonding and van der Waals bonds are responsible for the reactions of mucin and mucoadhesive polymers at the surface and the interfacial areas [10]. The interpenetration mechanism was directly supported by attenuated total reflection infrared spectroscopy and indirectly confirmed by the changes in rheological measurements of the mixtures of

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the two polymers [11–13]. Based on the rheological studies, the phenomenon of rheological synergism has been suggested as a measure of the degree of mucoadhesive interactions [13].

Studies on galacturonides and GIT epithelial tissues by rheological measurements have shown that pectin with low degree of esterification (DE) or having a large volume of linear oligogalacturonide segments possessed significant mucoadhesion against the GIT mucous membranes. Esterification, branching or heterogeneous backbone structures reduced the adhesive properties [5]. The molecular characteristics of pectins and other exogenous polymers play a key role on the stability of the resulting polymer–mucin gel structure, affect the bioactivity and bioavailability of incorporated substances, and alter the expression and physiological activity of endogenous organisms. Studies of the orientation and distribution of mucoadhesive polymers within mucin gels will have an impact on the design of gel formulations for both practical and basic research purposes.

In this study, high DE and low DE pectins as well as pectin derivatives carrying primary amine groups were evaluated for mucosal absorption and their distribution in mucus layer in vitro and ex vivo.

2. Experimental

2.1. Materials and sample preparations

Citrus pectins with the DE of 25% (P-25) or 94% (P-94), FITC-labeled bovine serum albumin (BSA) and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and used as purchased unless otherwise indicated. The DE of pectins was determined as described by Filisetti–Cozzi [14]. Pectin derivatives carrying side chain primary amine groups (P-N) were prepared from P-25 according to the method described previously using the coupling reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [15]. The amount of primary amine groups in P-N was measured directly by the TNBS method [16]. Fluorescence-labeled pectins were prepared by the conjugation of fluoresceinamine to the molecules of P-25, P-94, and P-N by Belder's method [17].

The pK_a values of P-25 and P-N were determined by potentiometric titration using a digital pH/milivolt meter (Model 611, Orion Research Inc., Cambridge, MA).

Pectin gels were prepared by dispersing pectin powders in phosphate buffered saline (PBS, pH 7.2) at the final concentrations of 2.5%, w/v. The mixtures were stirred at room temperature for 8 h and stored in a refrigerator overnight. For rheological analysis, the plain pectin gels were mixed with mucin by vortex at the highest speed; the mucin content in all mixtures was 2%, w/v. As a control, mucin dispersions at the concentration of 2%, w/v, in PBS also were prepared.

2.2. Rheological analysis

The viscosities of pectin or mucin preparations were determined by measuring the resistance to shear force, $73.2\,s^{-1}$ at 25 °C. A rotary viscometer, Cannon V-2000 series II (Cannon Instrument Company, State College, PA), was employed in this measurement.

The measurements of other rheological parameters were conducted using an AR series 2000 rheometer (TA instruments, New Castle, DE) equipped with a pair of aluminum parallel plates with a diameter of 2.5 cm. The test sample, 2.0 ml for each, was loaded on the lower plate evenly; the gap between the two plates was adjusted to 2.0 ± 0.04 mm and the sample was equilibrated at 37 ± 2 °C for 2 min. The linear viscoelastic region of test samples were determined by initial strain sweeps at a frequency of 10 Hz. The dynamic oscillatory test was performed at 37 ± 2 °C within the linear range. Storage modulus (G') and loss modulus (G'') were measured over a frequency range from 1 to 100 Hz at a fixed strain of 5%. The value obtained at the intermediate value of 10 Hz was chosen for further comparison and calculation because it is similar to the average value calculated over the linear range. From G' and G'' values the rheological synergism parameters, $\Delta G'$ and $\Delta G''$, were calculated as the difference between the actual viscoelastic values of the pectin/mucin test mixture and the sum of the values of the individual components [18]:

$$\Delta G' = G'_{(\text{mixture})} - [G'_{(\text{pectin})} + G'_{(\text{mucin})}]$$
$$\Delta G'' = G''_{(\text{mixture})} - [G''_{(\text{pectin})} + G''_{(\text{mucin})}]$$

2.3. Mucosal adsorption analysis

The ex vivo studies on the mucosal adsorption of pectins and incorporated proteins were conducted on the surfaces of porcine colonic mucus membranes. For pectin adsorption, a trace of fluorescence-labeled pectin was mechanically mixed with each pectin formulation prior to examination. The ratios of fluorescence-labeled pectin and non-labeled pectin in the gels were adjusted to reach the same level of fluorescence over all test samples using a fluorescence spectrophotometer (Model: Cary Eclipse; Varian, Walnut Creek, CA) equipped with a Xe flash lamp. The measurements were conducted at the excitation of 490 nm and the emission of 530 nm.

Porcine colonic tissues from freshly sacrificed pigs were obtained from a local slaughter house. The tissues were washed with tap water and transferred into

Waymouth's medium containing penicillin–streptomycin (100,000 units/L, 100 mg/L). The tissues were dissected into disk-shaped specimens with a diameter of 8.0 mm and used immediately. The tissue specimens were loaded with pectin formulations, 200 μl for each, and incubated under standard tissue culture conditions (CO_2 5%, O_2 95%) at 37 °C for 3 h. The formulations on the tissue surfaces were collected at desired time points using a micropipet and measured for fluorescence intensity to determine their pectin contents.

At the conclusion of incubation, the specimens were fixed in 2.5% glutaraldehyde solution for 3 h for structural analysis using confocal microscopy.

2.4. Microscopy analysis

2.4.1. Scanning electron microscopy (SEM)

For SEM examination, samples of pectin formulations and mucin gels as well as the pectin/mucin complex gels were dehydrated using gradient ethanol/PBS solutions, which was followed by critical point drying. Samples thus treated were mounted on specimen stubs, coated with a thin layer of gold by the use of a sputter coating apparatus (Edwards High Vacuum, Wilmington, MA). The coated samples were examined in a model JSM 840A SEM (Jeol USA, Peabody, MA) operating at 10 kV in the secondary electron imaging mode. Images were collected at 1000 × and 10,000 × using an Imix-1 digital image workstation (Princeton Gamma-tech, Princeton, NJ).

2.4.2. Confocal laser microscopy

Confocal laser microscopy was applied for the three-dimensional structure study of the pectin/mucin complex located in the colon tissue. Samples were glued to 1 × 3 in microscope slides and placed in the sample stage of an IRBE optical microscope with a 10 × lens integrated with a model TCS-SP laser scanning confocal microscope (Leica Microsystems, Exton, PA). The distribution of fluorescently labeled pectin within specimens of colonic tissues was visualized in sets of optical sections extending from the luminal surface into the intestinal wall. The parameters for the image acquisition were set for confocal fluorescence (488 nm excitation, 500–530 nm emission). Matching optical sections from different pectin treatments near the lumen and deep within the tissue were compared.

Autofluorescence induced by glutaraldehyde fixation was also visualized by confocal microscopy (488 nm excitation, 500–530 nm emission) and compared for the changes in tissue structure in series of optical sections (approximately 30–40 μm thick) visualized as a maximum projection image at low magnification.

2.5. Applications of pectin–mucin interaction

2.5.1. Pectin formulation as drug-delivery systems

For drug release study, BSA was used as a model protein drug and incorporated (together with a trace of FITC–BSA) into plain pectin gels at 5% (of the total solids) by mechanical mixing. The BSA release was conducted by the method described on Section 2.3, mucosal adsorption analysis. The amount of protein remaining in the pectin formulations after incubation was determined by measuring the fluorescent intensity at 490/530 nm.

2.5.2. Protective effect of pectin against toxic medium

Specimens of colonic tissues were pre-incubated with pectin formulations for 30 min under standard conditions. After the removal of free pectins on the tissue surfaces, the specimens were transferred into PBS solution containing 0.1% Triton X-100 and the incubation was continued for additional 30 min. The effect of the surfactant on the cellular structure of colon tissues was examined by confocal microscopy after fixing with glutaraldehyde. Specimens without pectin treatment were used as controls.

3. Results and discussion

3.1. Pectins

Table 1 shows the molecular characteristics of pectins used for this study. The low DE pectin, P-25, carrying high concentration of carboxylic acid groups, is considered a polyanionic polysaccharide. In comparison, the high DE pectin, P-94, is almost neutral. The carboxyl groups in P-25 were converted to primary amines to produce a polycationic polymer, P-N. The reaction is conducted by grafting ethylene diamine to the side chains of P-25 via amide linkages; thus, a group of hydrophobic $-CH_2CH_2$-segments at the concentration of 2.4 μmol/g was also introduced to P-N, as side-chain amines were generated. For all three pectin derivatives, hydroxyl groups are abundant. In addition, all preparations had similar viscosities, indicating that the macromolecules were closely comparable with

Table 1
Molecular characteristics of pectin derivatives

Samples	Functional groups (μmol/g)		pK_a	η
	$-COOH$	$-NH_2$		
P-94	0.20	—	—	0.99
P-25	2.40	—	4.2	1.03
P-N	—	2.5 ± 0.2[a]	9.3	0.94

[a]Data expressed as mean values (± SD); $n = 3$.

respect to the ratio of molecular weight to molecular size. Therefore, the variations of molecular weight among different pectin derivatives are ignored in the following studies.

Micrographs of pectin gels, P-94, P-25 and P-N, were obtained by SEM and shown in Fig. 1. All gels were composed of open porous networks, in which clusters or knots of entanglements were embedded. Strands in all gels were aligned rather than distributed individually; the strands bent randomly and often contain very sharp bends. Both P-94 and P-25 contained smooth strands connected through tri-functional and tetra-functional cross-links, but the strands in P-25 seemed looser and more flexible than in P-94; the strand lengths were smaller in P-94 than in P-25. The P-N gels had different structural composition, containing more bundles of aligned strands and densely packed fiber networks.

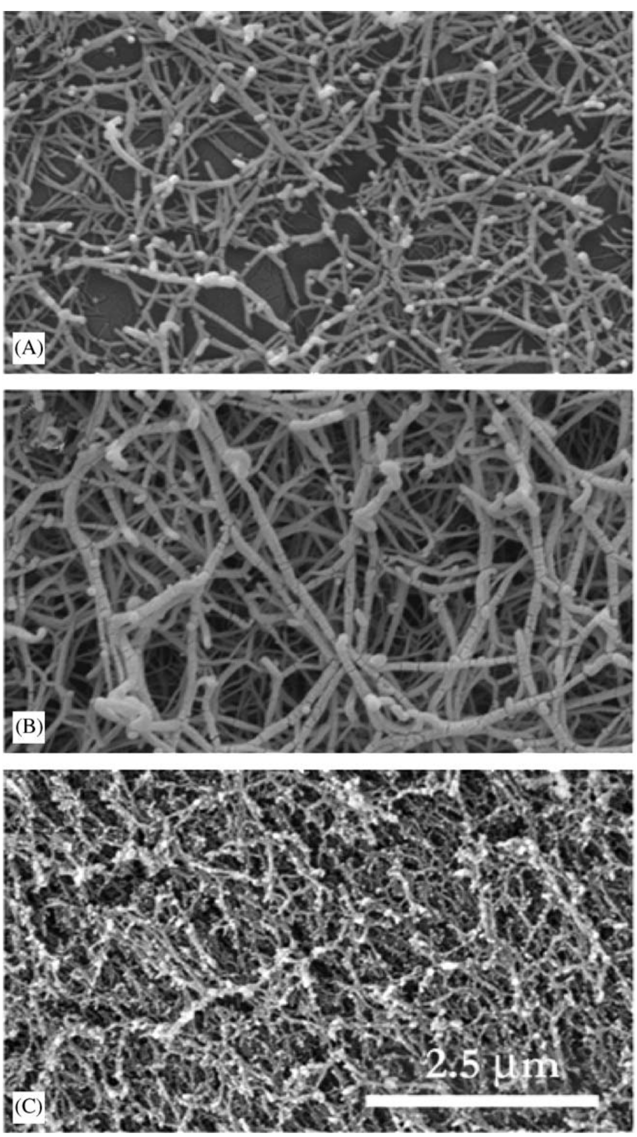

Fig. 1. SEM image of pectin derivatives. P-94 (A); P-25 (B); P-N (C). Bar = 2.5 µm.

Pectin network strands shown in this study have some similarities in shape and length to the aggregates shown by our previous work by atomic force microscopy (AFM) and transmission electron microscopy (TEM) [19,20], even though the networks presented in the current study were not induced by sucrose and were deposited from different solution concentrations. Thus, it appears that the pectin chains in solution are aggregated and have the tendency to form networks. The conformation of pectin molecules, which is a function of the type and density of side chain functional groups, eventually determines the microstructures of pectin gel networks.

3.2. Mixing of pectins with mucin

Commercially available porcine colonic mucin was used for this study because of its similarity in physiology, histology and structural properties with the human colonic tissue [21], and its low variability between batches [22]. Mucin samples for SEM examination were carefully dehydrated using a series of solutions of increasing ethanol concentration prior to critical point drying. The SEM images on Fig. 2A (low magnification) and B (high magnification) showed a typical three-dimensional network structure of mucin alone. These images confirm previously published observations found by TEM studies and AFM for other preparations of mucin [23–26]. Entanglement appears to be the dominate mode of mucin molecular association (Fig. 2A). All mucin gel preparations regardless of concentration showed lumps randomly associated with fibers to form a network (data not shown). Highly swollen regions of about 100 nm in size (Fig. 2B) corresponded to the dense "sugar coated" areas in mucin. These dense areas of sugar gave the mucin considerable water holding capacity. Microstructural images of porcine colonic mucin with added pectins are shown in Fig. 3. In general, pectin fibers were heavily obscured, showing evidence of internal binding with mucin aggregates (Fig. 3A–C). At the higher magnification, fibers and mucin aggregated into tightly bundled packs, which were attached to heavily entangled fibers (Fig. 3D–F). Regions of fiber-patch structures could be observed for the mixtures of mucin with P-94 or P-25. However, P-25/mucin composites produced a somewhat more homogeneous dispersion of mucin in the pectin networks. The two polymers appear to interpenetrate (Fig. 3B and E). Mixing P-N and mucin has a significant effect on the network structures of the individual polymers. Upon mixing, mucin seems to "coat" or "encapsulate" the P-N strands. P-N networks appear to provide a scaffold-like structure for mucin binding (Fig. 3C and F). We thus concluded that the adhesion of the two biopolymers occurred via the "swollen regions"

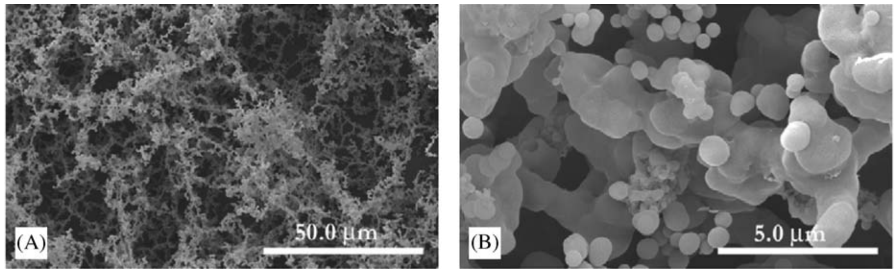

Fig. 2. SEM photograph of mucin at high magnification (A, bar = 50.0 µm) and at low magnification (B, bar = 5.0 µm).

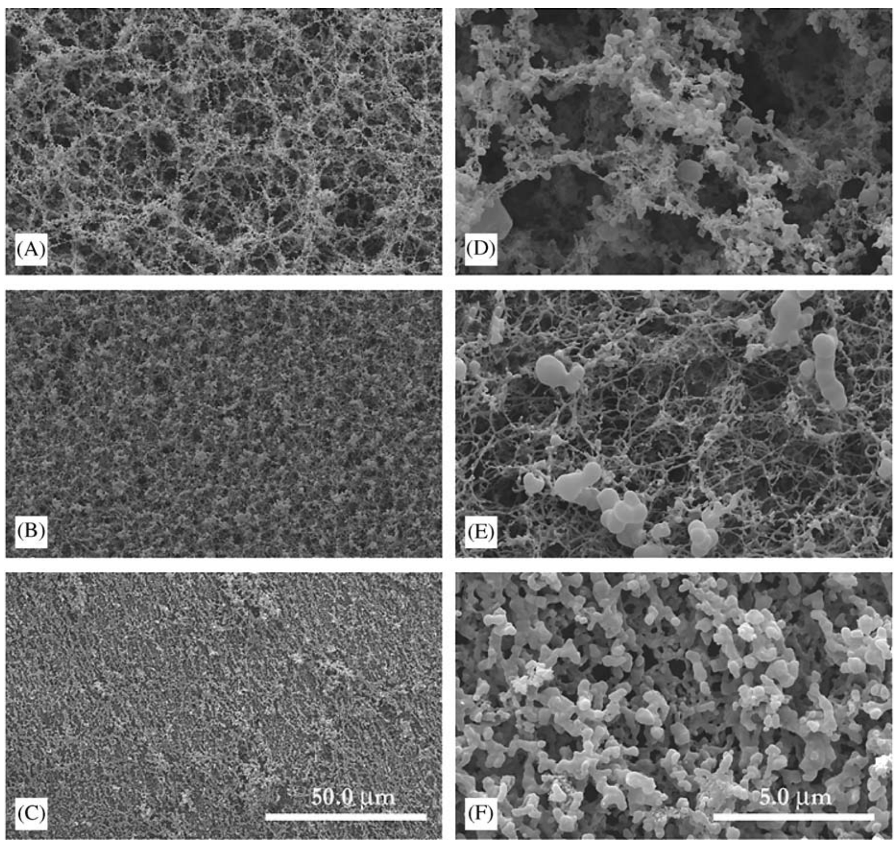

Fig. 3. SEM photograph of mucin gels with added pectin P-94 (A and D), P-25 (B and E), and P-N (C and F). (A)–(C): bar = 50.0 µm; (D)–(F): bar = 5.0 µm.

of mucin, as it was found for other cationic polymers such as chitosan and poly(vinyl pyridine) [24,26].

The differences in the structure of these complex gels can be attributed to the distinct characteristics of the pectins. At physiological pH, most of the carboxyl groups in pectin and the sialic acids of mucin are ionized. Therefore hydrogen bonding between pectin and mucin should be limited by electrostatic repulsion, whereas molecular entanglements should be favored due to expansion of coils. Hence, P-25, low DE pectin, seems to readily penetrate into the mucin networks with less difficulty than other pectins. In contrast, due to their relatively neutral electrical nature, the mobility of coiled P-94 chains is hindered by molecular entanglements, as evidenced by the formation of fiber-patches. These produce a heterogeneous gel structure. For P-N (pK_a of 9.3), the randomly distributed primary amine groups are mostly protonized at physiological pH. Electrostatic interaction is stronger than hydrogen bonding and chain entanglements. Supposedly, the interaction between P-N and mucin is initiated by the attraction of opposite charges, causing the glycoproteins to closely approach P-N fibers. The following hydrogen bonding and molecular entanglement strengthen the “coating” process. As the differences in three-dimensional microstructure will result in different viscoelastic properties of pectin and mucin gels, the viscoelastic properties of gel mixtures were investigated in the following section as a complement to microscopic imaging.

3.3. Gel rheology

Table 2 shows the viscoelastic properties of pectin and mucin gels alone. All gel formulations had higher G' value than G'' value, showing that their elastic tendency was greater than their tendency to flow. However, the value of tan δ for mucin preparation is higher than all pectin gels, indicating that mucin is less elastic than pectin gels.

The positive rheological synergism value(s) is evidence that dispersion of pectins reinforced the mucin gel structure (Fig. 4). The mucin/P-25 composites induced higher rheological synergism values than values from mucin/P-94 composite gels. The mixtures of mucin with P-N exhibited a more pronounced $\Delta G'$ and $\Delta G''$ than that obtained from the others (Fig. 4a). These differences suggest that more ordered gel networks were formed by the addition of P-N or P-25 into mucin rather than the use of P-94. The contribution of the three pectin derivatives to the viscoelastic properties of pectin/mucin formulations was further investigated by the calculation of their relative rheological synergism by dividing the dynamic moduli increase by the moduli of the individual polymers. The values of $\Delta G'/G'$ and $\Delta G''/G''$ express the contribution of each component to the rheological synergism effect. As shown in Fig. 4b, both the $\Delta G'/G'$ and $\Delta G''/G''$ values for all preparations confirmed the trend observed for other rheological synergism parameters. In particular, the values of $\Delta G'/G'$ and $\Delta G''/G''$ of P-N showed the highest relative increase in comparison to P-25 and P-94 gels, indicating that the introduction of positively charged polymers tends to form a rigid gel with mucus glycoproteins. The results from small deformation rheological measurements are consistent with microscopic analysis for gel three-dimensional microstructural studies (Fig. 3), where a tightly compact fiber structure was found for the gels complexing with P-N; an interpenetrating network with P-25 and the reinforced networks of randomly dispersed fiber-patches for gels with P-94 were shown.

Table 2
Viscoelastic properties of pectins and mucin

Samples	G' (Pa)	G'' (Pa)	tan δ
P-94	14.33 ± 2.74	2.77 ± 0.89	0.193
P-25	17.35 ± 2.11	3.44 ± 0.56	0.198
P-N	11.95 ± 3.55	4.46 ± 1.12	0.373
Mucin	2.73 ± 0.04	1.44 ± 0.14	0.533

Data expressed as mean values (±SD); $n = 3$.

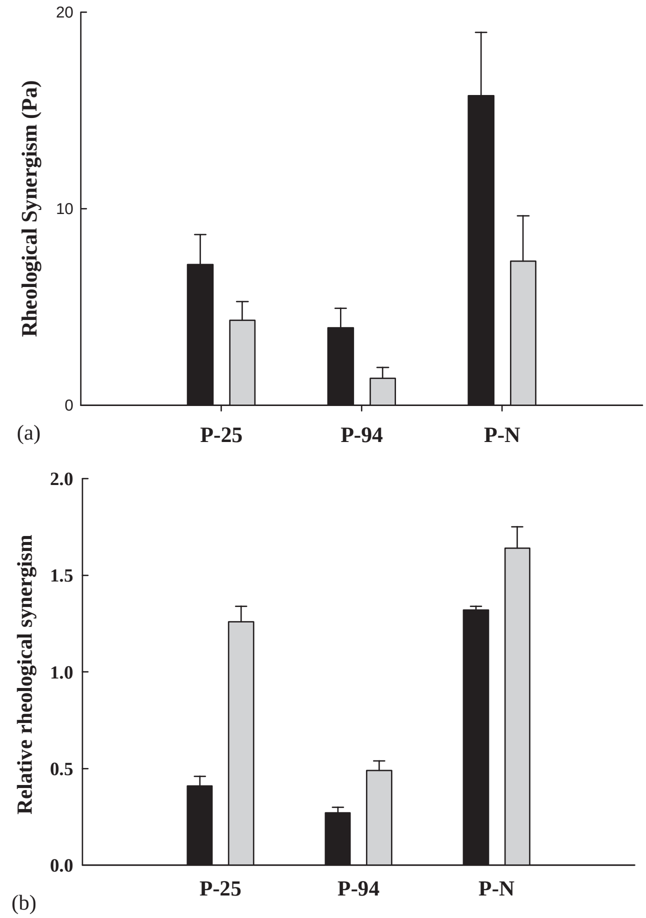

Fig. 4. (a) Rheological synergism of various pectin and mucin mixtures. $\Delta G'$: black column; $\Delta G''$: gray column. (b) Relative rheological synergism of same mixture. $\Delta G'/G'$: black column; $\Delta G''/G''$: gray column.

The extent of rheological synergism of different pectin and mucin gel networks may be related to response of structural features of pectin derivatives. The increase in $\Delta G'$ and $\Delta G''$ in pectin–mucin mixtures is attributable to the creation of more network links or the replacement of weaker chains entanglement by stronger chain–chain association [18,27]. Previous studies have shown a significant mucus gel strengthening by incorporating known strongly mucoadhesive polymers such as PAA [13,26,28] or chitosan [24]. Polyelectrolyte polymers produce rheological synergism with mucin by different mechanisms, depending on their molecular characteristics. The positive charges carried by P-N increase gel strength significantly. As the rheological analysis and microstructural investigation suggested, electrostatic attractions produce ionic bonding and facilitate hydrogen bonding and chain entanglement; electrostatic repulsions limited hydrogen bonding. Nevertheless, electrostatic repulsions induced relatively homogeneous structure and made the higher molecular weight polymers more susceptible for chain entanglements. These last two effects account for strengthening the gel structures, as seen for P-25-induced mucin gels.

3.4. Ex vivo study of pectins with porcine colonic tissue

The above experiments were based on mechanical mixing of mucin gels with different pectin formulations. It provides information on the reaction of pectin with mucin at the molecular level. However, mechanical mixing is not likely to occur in vivo. Alternatively, a diffusion model has been suggested [26,5]. Fig. 5 shows the time curve of pectins adsorbed by the porcine tissues. There is a sharper initial decrease in FITC intensity for all pectin formulations in the first 5 min; and the amount of pectin remaining gradually decreased for the 3 h which followed. The maximum of pectin adsorption was dependent on which pectin was used. More pectin was adsorbed by specimens treated with P-N than those treated with P-25 or P-94.

The distribution of adsorbed pectin in the mucin layer was investigated next using confocal fluorescent scanning microscopy. The results were obtained from side-views of optical section stacks made through the surface of the lumen to deep within the wall with an average depth of 180 μm (Fig. 6). The peak position of P-94 was near the lumen whereas the peak intensity of P-25 was very deep into the wall. At the lumen area, there was more P-94 accumulated than P-25 gel. The peak of the P-N distribution curve also appeared near the lumen position but its intensity was as high as that of P-25 in the wall area. These results (Figs. 5 and 6) confirmed the conclusion drawn from the in vitro experiments by the mixing model, which showed a higher activity of the negatively charged P-25 in penetration into the mucin. P-N is more susceptible to ionic binding, thus it is easier for P-N to form a stronger network with mucin upon contact. The high DE pectin, P-94, remaining in a hydrocolloid state, is easy to be entrapped in the mucus

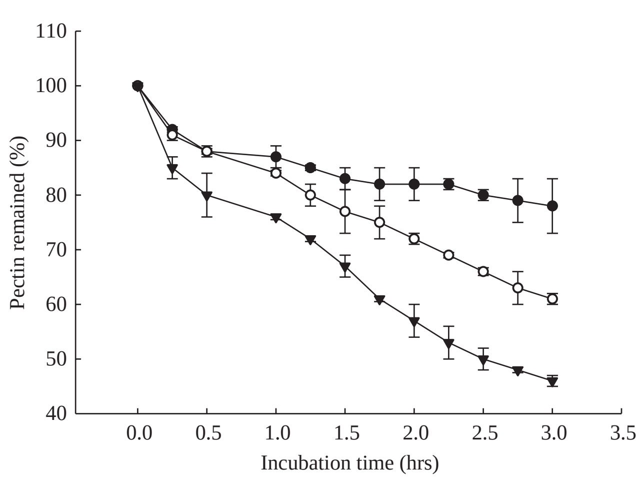

Fig. 5. Absorption of pectin from pectin formulations by porcine colonic mucus tissues. P-94 (●), P-25 (○), and P-N (▼).

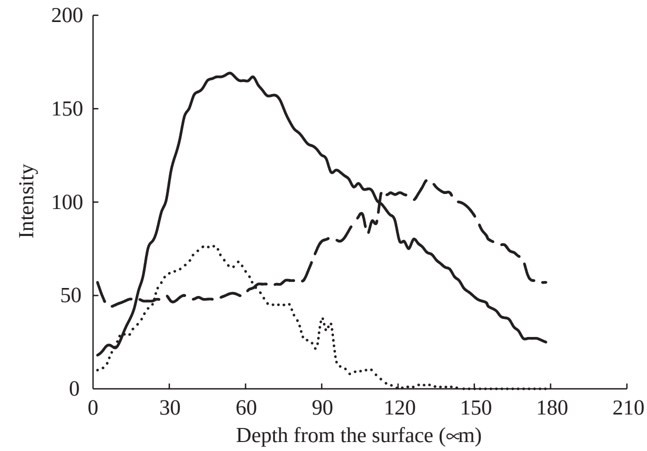

Fig. 6. Fluorescent intensity of pectin formulations extending from the surface of the lumen to deep within the wall made through the stacks of optical sections. P-N (solid line), P-25 (broken line) and P-94 (dotted line).

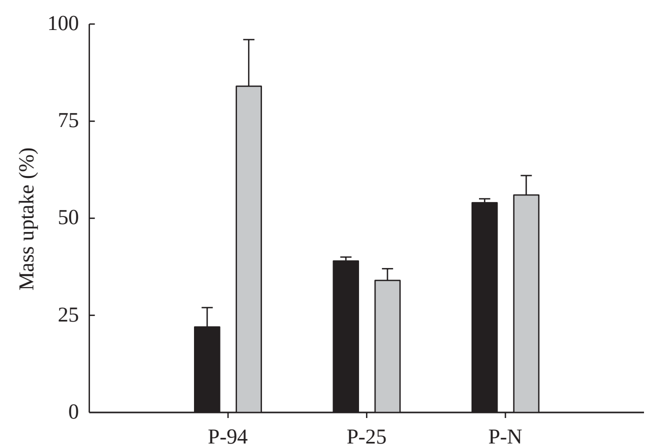

Fig. 7. Comparison of pectin uptake (black bar) and BSA uptake (gray bar) from various pectin or pectin/protein formulations by porcine colonic mucus tissues.

to form complex gels; but this, in turn, reduces the mobility to penetrate deeply into the mucus layer.

3.5. Applications of pectin–mucin interaction

3.5.1. Pectin formulations as drug-delivery systems

BSA was used as a model drug to investigate the mechanism, by which drugs are released from various pectin formulations. To simplify the experimental system, the effect of bacteria and enzymes on pectin degradation was ignored. As shown in Fig. 7, the amount of adsorbed protein differed among the pectins investigated. These differences could be rationalized by considering gel–tissue interactions. For P-25 and P-N gels, pectin and BSA adsorption had similar ratios. In contrast, the release ratio of BSA from P-94 formulation was much higher than the ratio of pectin adsorption. We concluded that the release of BSA from P-94 was diffusion controlled, while the release of BSA from P-25 and P-N seemed mainly to be determined by the pectin dissolution with mucus tissues.

Due to its benefits the oral delivery of bioactive materials including vaccine protein(s) via the intestinal mucus membrane is gaining increased attention [29,30]. The immunization can be performed without injection and without medical personnel; the delivery with carrier reduces the number of doses and the length of time required to stimulate full protection. Oral immunization stimulates IgA immunoglobulin which is not induced by parenteral immunization. Oral immunization provides a first line of topical local defense against the threat of the invading microorganism or toxins of microbial or plant origin. The topic and the results presented in this manuscript establishes a rational approach to construct a vaccine carrier suitable to adhere and penetrate through the mucosal membranes of the alimentary tract in an environment with short residence time unlike the conditions of parenteral administration. While much attention is being devoted to the optimal biadhesion of a carrier, the optimal penetration potential of the polymeric carrier has attracted virtually no attention. Mucoadhesive polymers other than pectin that were investigated include PAA, CMC, HA, chitosan, gelatin and polyethyleneglycol (PEG). PEG has a well-established bioadhesive function in the process of fusing T cells into myeloma protein to obtain monoclonal antibodies. PEG is also used to coat nanoparticles made of polylactic acid (PLA) for the delivery of tetanus toxoid nasal vaccine [31]. It has been revealed that PEG coating of PLA nanoparticles had a role in stabilizing PLA particles in the uptake of mucosal fluids. Nevertheless, its penetration potential relative to other bioadhesive polymers has not been explored. Furthermore, the penetration potential of other mucoadhesive polymers has not been investigated.

The high penetration potential of P-25 and P-N and their high rheological synergism value favor their selection for the delivery of vaccines across mucosal barriers. Although it is difficult to predict a priori whether P-25 or P-N will be a better carrier system for oral vaccine(s) delivery, on the basis of SEM images, P-N appears to be more suitable. SEM images revealed that P-N had more aligned bundles of strands than either P-25 or P-94. Consequently, P-N possess higher contact area for interaction with the mucous membrane than either P-25 or P-94. As a result formulations made with P-N have firmer interactions with mucosal membranes than P-25 or P-94. Firm mucosal interactions are a requisite for the increased residence time needed to accomplish a biological process in an environment where mechanical and chemical clearing forces act on every foreign entity.

3.5.2. *Pectin formulations as protective barriers*

It has been reported that rhammogalacturonan can protect colon tissues from destruction by toxic substances [5]. In the current study, the protective effect of three pectin formulations on cellular destruction was compared. The pattern of autofluorescence in the colon was used to compare the protective effect of various pectin gels on the changes in the intestinal wall induced by exposure to Triton X-100. These results were illustrated in Fig. 8. In normal, untreated specimens, the fluorescence in maximum projection images of the colon wall revealed the cellular distribution within tissue. Fig. 8A is an image of the colon wall prior to treatment with Triton X100. In that image we observe that epithelium lines the lumen, and that luminal pockets are separated by spaces of cellular submucosal tissue. In Triton X-100 treated specimens, the distribution of fluorescence was very different from normal tissue; most of the fluorescence was located in the epithelium lining the luminal pockets, and the fluorescence in submucosal tissues is homogeneous and diffused (Fig. 8D). Two of the specimens pretreated with pectin gels, P-94 and P-N (Fig. 8B and C), revealed a pattern of fluorescence similar to normal, untreated tissue specimens (Fig. 8A), but the specimen treated with P-25 (Fig. 8E) has a fluorescence pattern that more closely resembles the Triton X-100 treated specimens (Fig. 8D), indicating that the pectin gels, which form networks with mucin on the surface of the colonic tissues, can exert a stronger protective effect towards exposure of the colon wall to an irritant.

4. Conclusions

In this study, we evaluated the possibility of using various pectins to manipulate the architectures and properties of colon mucosal tissue. SEM and small deformation rheological studies suggested that pectins with higher net charges were more mucoadhesive than the other pectins. Pectin uptake could be enhanced either by lowering the degree of esterification or by replacing side chain carboxyl groups with primary amine groups. The release of incorporated bioactives from P-N and P-25 formulations was found to be determined by pectin dissolution, while the release of drugs from P-94 gels was controlled by a diffusion mechanism.

Furthermore, high DE pectin formed gel networks with endogenous mucin lining on the surface of the mucosal tissues. Pectin derivatives carrying positive charges also form thicker gels on the tissue surface. The mucin–gel complex formed between P-N or P-94 and mucus tissue constructs a surface barrier which effectively prohibited the penetration of Triton X-100. Low DE pectin was able to penetrate deeply toward the

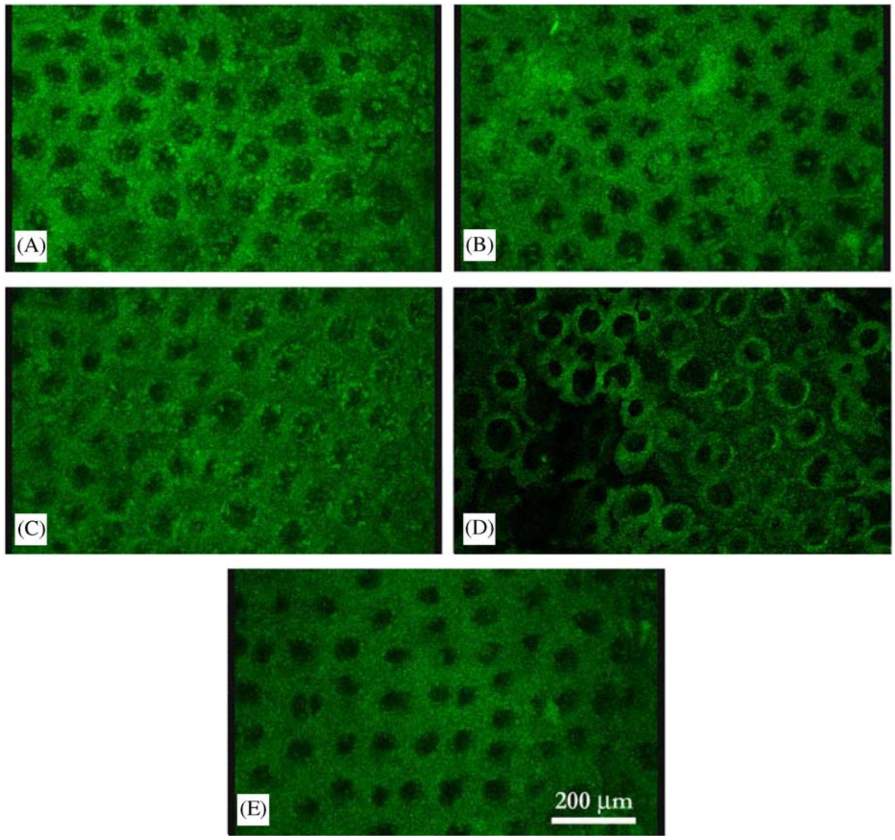

Fig. 8. Images of porcine colon tissues (A) before and (D) after exposure to Triton X100. Prior to triton X100 exposure, porcine colon tissues were treated with pectin gels of (B) P-94, (C) P-N, and (E) P-25.

intestinal wall, but did not adhere strongly on the tissue surface. Thus, P-25 gel did not inhibit Triton X-100 irritation of the mucus tissue as effectively as the gel formed by P-N/mucus complex.

By changing the molecular characteristics of pectin, we were able to alter the strength of pectin and mucus gel networks and the pattern of pectin distribution in the pectin/mucin complex. Thus, it is possible to guide the interaction of pectin with mucin in situ to satisfy specific requirements of drug delivery and clinical therapeutics.

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